Toxicokinetics: An Analytical Tool for Assessing Chemical Hazards to Man

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The Toxic Hazards Division has pioneered the development of toxicokinetic analysis for the study of the toxicity of various chemicals of importance to the U.S. Air Force. *Toxicokinetic analysis* permits calculation of tissue exposure based on biochemical, physiological, and physical chemical properties of the animal-chemical system. This paper describes the application of toxicokinetic analysis in the study and control of chemical hazards. Physiological models for both carbon tetrachloride and methylene chloride are discussed in relation to their ability to predict human kinetics and their use in estimating the risk of these chemicals to exposed humans. The emerging use of the toxicokinetic approach to analyze the mechanistic basis of chemical carcinogenesis is also discussed.

ACCURATE, TIMELY estimates of the human hazards associated with occupational and environmental exposure to chemicals play an important role in every aspect of U.S. Air Force (USAF) operations. The requirements are many and varied. Are current occupational exposure limits for fuels and solvents adequate to protect the nursing infants of working mothers? Does a new seat cushion material have a potential to asphyxiate aircrew in the event of an aircraft cabin fire? Must the ground crew working with hydrazine-fueled emergency power units wear cumbersome protective suits that make maintenance operations difficult or will respirators alone provide sufficient protection? Does the contaminated groundwater near a particular base pose a serious threat to human health, or should first priority be given to some other hazardous waste site containing a different combination of chemicals? To answer these and similar pressing questions, the Toxic Hazards Division of the Harry G. Armstrong Aerospace Medical Research Laboratory (AAMRL) maintains a broadly based and responsive research program in toxicology designed to identify and quantitate health hazards associated with fuels, chemicals, and materials used in current and future USAF systems and operations.

Except in the rare instance when an adequate epidemiological data base is available, safe human exposure levels for chemicals and fuels must be based on extensive animal testing. These traditional animal experiments are time-consuming and expensive and have come under increasing criticism. More importantly, much animal data cannot be directly extrapolated to man because differing physiological and biochemical factors result in dramatically varying responses to the same chemical. The deficiencies in the approaches to hazard assessment stimulated our research in analytical modeling techniques to reduce reliance on large-scale animal experiments and provide a more systematic approach to hazard evaluation. This approach, called toxicokinetics at AAMRL, is used as an analytical tool for predicting the time-dependent uptake, distribution, metabolism, and excretion of potentially toxic chemicals and their metabolites in the body. Using toxicokinetic analyses in combination with mechanistic studies, the amount of a toxic agent reaching a target organ or tissue can be inferred from chemical exposures of test animals. Hazard assessment then simply involves determining the exposure concentration that yields tissue levels in man similar to those causing overt toxicity in test animals. This analytical approach can help toxicologists and other health professionals to rapidly assess the hazards of a chemical, efficiently design necessary animal experiments, and accurately extrapolate animal data to man.

Physiological Modeling

The basic approach in toxicokinetic analysis is illustrated in Fig. 1. A physiological toxicokinetic model

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uses basic mechanistic, biochemical, and physiological information to describe the uptake, distribution, and disposition of a chemical in the body. Mechanistic studies provide basic information on the target tissues, metabolic pathways, and nature of the toxic species: parent, stable metabolite, or reactive intermediate. Biochemical data consist of partition coefficients for the chemical in blood and tissues as well as metabolic constants and permeation coefficients. Physiological factors include organ and tissue volumes, blood flow, and ventilation rates. One of the real advantages of physiological modeling as compared to conventional compartmental models is that much of this information can be gleaned from the literature or acquired using *in vitro* techniques, greatly reducing dependence on animal experiments.

Using this information, the investigator develops a physiological model which expresses mathematically his conception of the animal-chemical system. In an iterative process the model is then exercised, its predictions are compared with experimental data, and the model is refined as necessary. The model development process also provides an opportunity to design those critical experiments which can provide data needed for verifying or improving model performance. The final product of this process is a validated model for the chemical of interest. Because of its physiological basis, the validated model can then be used to extrapolate across routes of exposure and between species by simply changing the values of the appropriate parameters.

In formulating a physiological model the body is described as a set of biologically explicit compartments—the liver, kidneys, lungs, gut, fat, etc. Each compartment has defined volume, blood flow, partition coefficient, and metabolic constants. The model can often be simplified by grouping certain tissues into single compartments, for example, well perfused, moderately perfused or poorly perfused tissue groups. The model shown in Fig. 2 was designed to analyze the inhalation of gases and vapors (6); however, other classes of chemicals and other routes of uptake can easily be conceptualized in the same manner. Until the last two decades, it was difficult to mathematically solve the differential

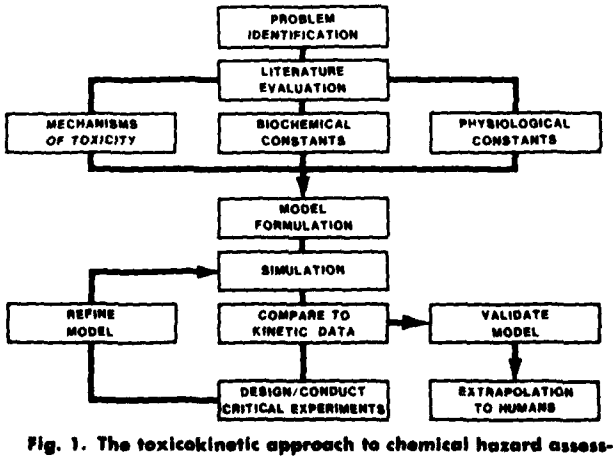

Fig. 1. The toxicokinetic approach to chemical hazard assessment.

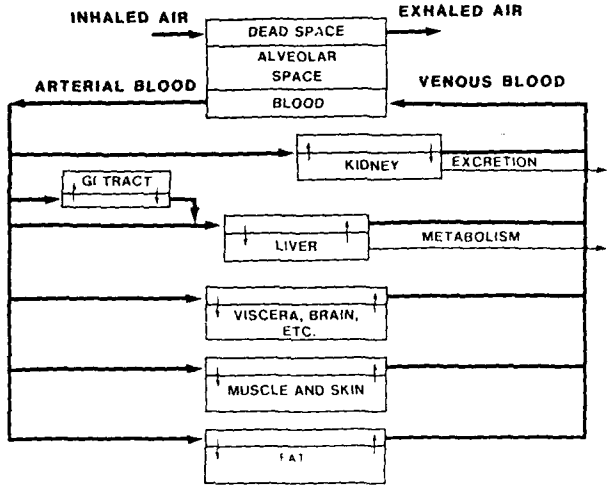

Fig. 2. A physiologically-based pharmacokinetic model for inhaled vapors.

equations describing the time-dependent uptake, movement, and metabolism of a chemical in a physiological model. Researchers then used simpler, one- or two-compartment models which were amenable to rigorous mathematical solution. The availability of sophisticated simulation packages capable of numerically solving large numbers of mass-balance differential equations now makes the manipulation of these complex models routine. Using these simulation languages, we have developed a hierarchy of models for the laboratory. These range from research versions for examining changes in the overall structure of the model to user-friendly versions that provide laboratory personnel unskilled in computer programming with a simple tool for prediction and analysis of experimental results (2).

Example: carbon tetrachloride: As an example of the power of the physiologically based toxicokinetic modeling approach, we have used our physiological model for inhalation of vapors to describe the kinetic behavior of carbon tetrachloride (CCl_4) in both rodents and man (5). The question addressed by this particular experimental study was whether a change in workshift schedule from a standard 5-d, 8 $h \cdot d^{-1}$ workweek to a nonstandard schedule involving longer shifts would affect the cumulative burden of carbon tetrachloride in exposed workers. Conventional analysis of the data, which was obtained with rats, did not provide any indication of a significant difference between the two exposure scenarios; however, there was concern that the results for rats might not adequately reflect the impact on humans. To provide a more thorough analysis, we developed a physiological model for carbon tetrachloride in the rat and compared its predictions with the experimental data. The prediction of the model for the concentration of carbon tetrachloride in expired air for several days following the last exposure period in a 2-week study is shown as the solid line in Fig. 3. The actual concentrations measured for the rats are shown as individual symbols.

The next step in the analysis was to confirm the model's ability to predict the kinetics in humans as well.

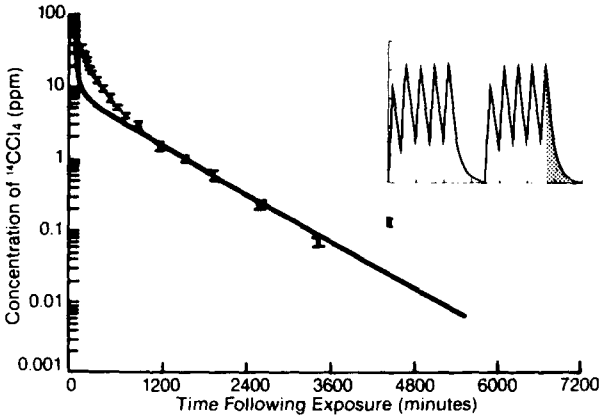

Fig. 3. Comparison of model predicted (solid line) and actual concentrations (bars indicate standard error) of carbon tetrachloride (CCl_4) in the expired breath of rats following exposure to 100 ppm CCl_4 for 8 h·d^{-1} for two 5-d periods separated by a 2-d interval.

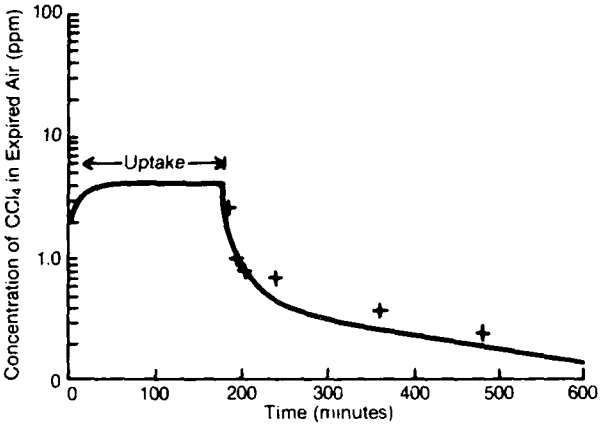

Fig. 5. Comparison of model predicted (solid line) and actual (+) concentrations of CCl_4 in the expired breath of human volunteers exposed to 10 ppm of CCl_4 for 180 min. Data from Stewart et al. (7).

Because of its physiological basis, all that was required to make the model predict human kinetics was to change the physiological and biochemical parameters from those measured for a rat to those established for an average human. The model was then exercised for two exposure scenarios for which experiments with human volunteers had been reported in the literature. Without any adjustment of the model parameters to try to fit the human data, the predictions of the model were simply plotted together with the actual experimental points as a test of the model's ability to perform interspecies extrapolation. The results are shown in Figs. 4 and 5. The first case (Fig. 4) is a 70-min exposure to 49 parts per million (ppm) of carbon tetrachloride. Again, the model prediction of expired air concentration is the solid line and the data for human volunteers are the individual points. The second case (Fig. 5) is a 3-h exposure to 10 ppm. In both cases, the excellent agreement of the model predictions with the human data gives us increased confidence in the ability of the model to provide a sound basis for using animal data to estimate the hazard to humans.

By its design, the physiological model automatically keeps track of a number of interesting quantities. The predictions of the model for the concentration of carbon tetrachloride in the fat tissue of rats during 2 weeks of repeated exposures are shown in Fig. 6. The symbols reflect the concentrations actually measured in fat tissue obtained from rats in the experimental study. Taking advantage of the ability of the model to extrapolate across species, we can now ask whether the expected behavior in humans would be different from that observed in rats. As can clearly be seen in Fig. 7, the model predicts that the loading of carbon tetrachloride in the fat tissue of humans during a workweek is considerably different from that observed in the rat. The more rapid clearance of the chemical in the rat prevents accumulation from day to day, whereas the much slower time-constant for humans leads to a different burden of chemical at the end of the week than earlier

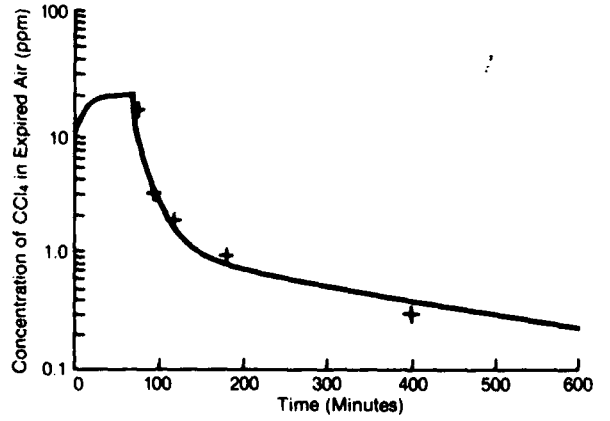

Fig. 4. Comparison of model predicted (solid line) and actual (+) concentrations of CCl_4 in the expired breath of human volunteers exposed to 49 ppm of CCl_4 for 70 min. Data from Stewart et al. (7).

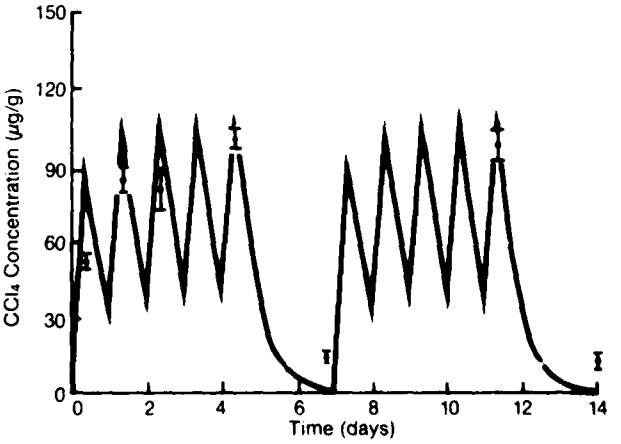

Fig. 6. Comparison of model predicted (solid line) and actual concentrations (bars indicate standard error) of CCl_4 in the adipose tissue of rats exposed to 100 ppm CCl_4 for 8 h·d^{-1} for two 5-d periods separated by a 2-d interval.

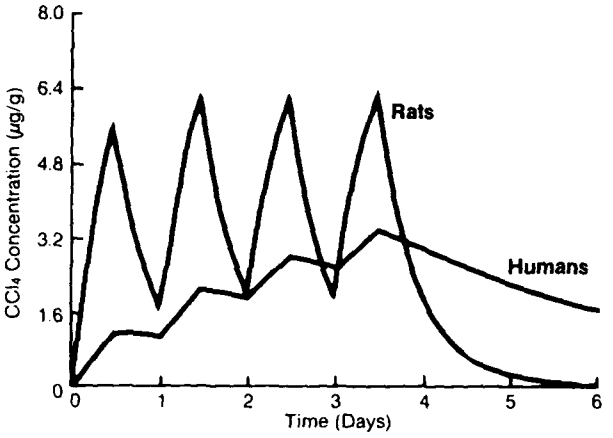

Fig. 7. Comparison of model predicted concentrations of CCl_4 in the adipose tissue of rats and humans exposed to 5 ppm CCl_4 for 11.5 h for 4 d.

on. This difference has a major impact on the proper interpretation of the animal experiments. Whereas the results in rats showed little effect of the change in work-shift schedule, by using the physiological model it becomes evident that significant differences can be anticipated for human workers.

Metabolite Modeling

To adequately interpret the toxicity of some chemicals it may be necessary to include a description of their metabolites in the model. The principles of the physiological approach are unchanged in this case, but additional data must be collected to characterize the metabolic pathways. An example of a successful model of this type is our description of the dihalomethanes (3). This model (Fig. 8) has been applied to dibromomethane, bromochloromethane, and methylene chloride, among other chemicals. The model not only describes the uptake, distribution, metabolism, and excretion of the parent chemical, but also follows the production of carbon monoxide by a capacity limited, oxidative metabolic pathway, the binding of the carbon monoxide to hemoglobin in the blood, and the clearance of unbound carbon monoxide by the lungs. The model also calculates the release of halide ion by both the oxidative pathway and a second pathway involving conjugation with glutathione. This model has successfully described the time course of the parent chemical, of blood carboxyhemoglobin, and when appropriate, of bromide ion, for inhalation, intravenous, oral, and dermal exposures under a wide variety of exposure conditions, and in several different species. Two examples of the agreement of the model's predictions with experimental measurements are shown at the bottom of Fig. 8.

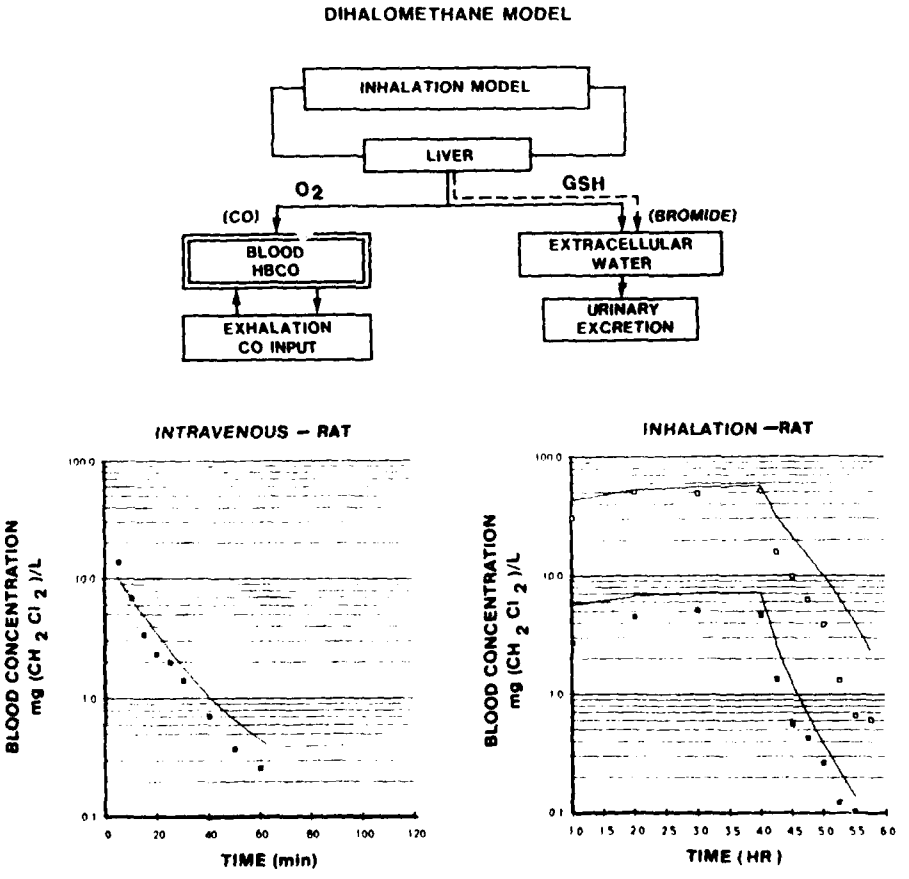

Fig. 8. Schematic of a physiologically based pharmacokinetic model for dihalomethanes (top panel), and comparison of model predicted blood concentrations of *methylene chloride* (CH_2Cl_2) in rats (solid lines) with *actual* data for a 10 mg·kg^{-1} intravenous injection of CH_2Cl_2 (■, left panel) and for 4-h inhalation exposures to 200 ppm (■) and 1000 ppm (□) CH_2Cl_2 (right panel).

On the left is shown the predicted and observed time course of methylene chloride in the blood of rats following an intravenous dose of 10 $mg \cdot kg^{-1}$. On the right are similar comparisons for two inhalation exposures to methylene chloride. These comparisons are 4-h exposures at 1,000 ppm (higher curve) and 200 ppm (lower curve).

Another example of the remarkable capability of the model is shown in Fig. 9. In this case, the chemical being modeled is methylene chloride, and the experiment is a 6-h inhalation exposure performed with human volunteers at the Dow Chemical Company some years ago. On the left are the model predictions and experimental observations for the concentration of methylene chloride in the blood, during and following the exposure period. On the right are the predicted and measured concentrations of carbon monoxide in the exhaled air. We have recently applied this model to perform a toxicokinetic risk assessment for methylene chloride (1). This marks the first time a chemical risk assessment has been performed which considers quantitatively the impact of species differences in the uptake, distribution, metabolism, and excretion of the chemical when calculating the estimated human risk.

Methylene Chloride Risk Assessment

Methylene chloride is an important USAF solvent and a common groundwater contaminant that has recently been found to be positive in an animal carcinogenicity bioassay. Since we had already constructed a working physiological model for methylene chloride, we were in an excellent position to impact the rule-making process for a chemical of major interest to the U.S. Air Force while demonstrating the usefulness of the toxicokinetic approach. One of the crucial steps in the toxicokinetic risk assessment is the determination of the appropriate measure of dose to use in extrapolating from the tumor incidence found in the animal studies to the tumor incidence expected for human exposure levels. Methylene chloride itself can be ruled out as the tumorigenic agent on mechanistic grounds. As seen in Table I, the tissue dose calculated by the model for products of the glutathione-dependent pathway correlates well with the corresponding tumor incidence in the inhalation studies, while the tissue dose calculated for products of the oxidative pathway does not. From this and other evidence, the tissue dose from the glutathione-dependent pathway was chosen as the metric for the risk calculation.

TABLE I. METHYLENE CHLORIDE BIOASSAY RESULTS AND TISSUE DOSE CALCULATION FOR FEMALE B6C3F1 MICE.

	Control	Inhalation (2,000 ppm)	Inhalation (4,000 ppm)
Liver			
Tumors (%)	6.0	33.0	83.0
O_2 Pathway	0.0	3,575*	3,701
GSH Pathway	0.0	851*	1,811
Lung			
Tumors (%)	6.0	63.0	85.0
O_2 Pathway	0.0	1,531	1,583
GSH Pathway	0.0	123	256

* Relative units for tissue dose

The results of the toxicokinetic approach are compared with the conventional, linear extrapolation approach in Table II. The toxicokinetic analysis suggests that the conventional approach overestimates the human risk from methylene chloride by roughly a factor of 100. The biological motivation for this result is that the conventional approach ignores interspecies differences in metabolic capacity and the nonlinear behavior of the metabolism of methylene chloride. At the very high exposure concentrations needed for the animal bioassay, the oxidative pathway is saturated and significant concentrations of methylene chloride are available in the liver for the glutathione-dependent pathway. However, at the very low concentrations typical of human exposure, the oxidative pathway is not saturated and is able to compete effectively for a much greater share of the

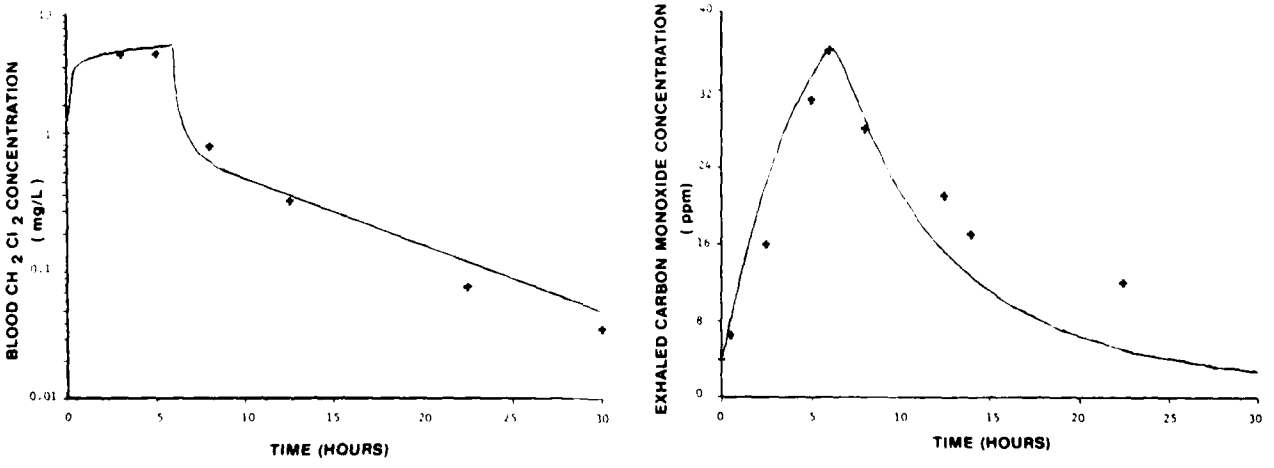

Fig. 9. Comparison of model predicted (solid line) and actual (+) concentrations of CH_2Cl_2 in the blood (left panel) and carbon monoxide in exhaled breath (right panel) of human volunteers exposed to 350 ppm CH_2Cl_2 for 6 h.

TABLE II. CANCER RISK ASSESSMENT BASED ON TARGET TISSUE DOSE CALCULATIONS.

	Conventional Approach	Toxicokinetic Approach	Ratio
Mouse-Inhalation 4000 ppm			
Liver	1,811*	1,811	—
Lung	255*	255	—
Human-Inhalation 1 ppm			
Liver	1.34	0.0080	167
Lung	0.19	0.0013	144
Human-Drinking Water 1 mg · L^{-1}			
Liver	0.211	0.0047	45
Lung	0.029	0.00014	213

* Relative units for tissue dose

total metabolism. Thus, the oxidative pathway is protective in this instance.

As a result of the noticeable discrepancy between the predictions of the toxicokinetic approach and those of the conventional risk assessment, the U.S. Environmental Protection Agency has halted its rule making on methylene chloride to consider incorporation of toxicokinetic techniques into their own risk assessment process. The potential impact on the USAF Installation Restoration Program is far reaching and is not limited to methylene chloride. Although less stringent remedial action levels could greatly reduce site clean-up costs, the most important potential benefit is the better protection of public health by providing more accurate risk estimates for use in identifying those sites that present the most serious hazard.

Future Research

We are continuing to expand our research efforts in toxicokinetics, particularly in the modeling of pharmacodynamics; that is, in modeling not only the movement of the chemical in the biological system, but also the response of the biological system to the presence of the chemical. One of the most challenging modeling areas, and one toward which we are now directing considerable effort, is the process of chemical carcinogenesis. There are actually several processes which have been described by which different chemicals appear to produce tumors. Methylene chloride appears to be an example of a genotoxic carcinogen; that is, a chemical which exerts its effect through the direct chemical interaction of itself or one of its metabolic products with genetic structures of the cell. Other possible mechanisms involve cell toxicity, in which increased cell turnover indirectly affects the likelihood of mutation, and promotion, in which exposure to a chemical increases the likelihood that a precancerous cell will go on to form a tumor. Whatever the suggested mechanism, if it can be described, it can be modeled. For example, all of the

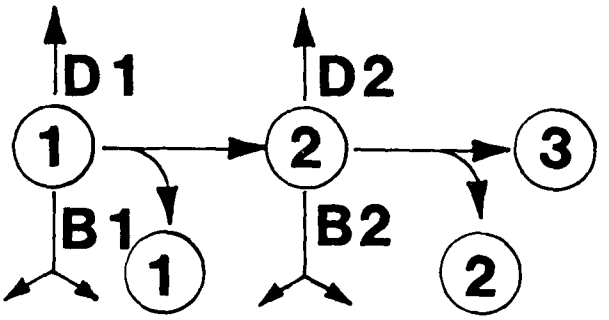

Fig. 10. A two cell-stage cancer model consisting of normal cells (1), precancer cells (2), and cancer cells (3) with appropriate birth rates (B1, B2) and death rates (D1, D2).

mechanisms just mentioned can be described in terms of the model (Fig. 10) proposed by Moolgavkar and Knudson (4). The challenge is to define the functional relationship between the chemical dose to the tissue and the parameters in the pharmacodynamic model.

In summary, what began as a small, hopeful basic research program has grown in just a few years into a methodology and philosophy which now underpins every aspect of our work, and which may well help to revolutionize the entire field of toxicology. Toxicokinetic modeling can improve experimental design of toxicity studies by allowing the investigator to identify the administered dose which will produce the required dose delivered to the target organ. As shown in the methylene chloride risk assessment, toxicokinetic modeling can also be used to calculate potential correlates of toxicity as an aid in interpreting toxicity studies and to provide a more rational approach to chemical risk assessment than those currently used. At the same time, we are expanding our exploratory efforts into the development of pharmacodynamic models of carcinogenesis, we have also created a new branch in the Toxic Hazards Division especially to explore applications of the currently developed modeling techniques. This branch is structured to address pressing, contemporary USAF concerns such as transfer of occupational chemicals to nursing infants, uptake of toxic vapors through the skin, safe levels of chemical agents in shelters, biological monitoring of workplace exposures, and interaction effects on the toxicity of mixtures of chemicals present in groundwater. Perhaps the most important feature of the toxicokinetic approach is that the physiological model provides a conceptual framework with which the toxicologist can more effectively conduct the scientific method: generating quantitative, testable hypotheses, designing appropriate experiments, and revising the theory (model) on the basis of discrepancies between theory and observation.

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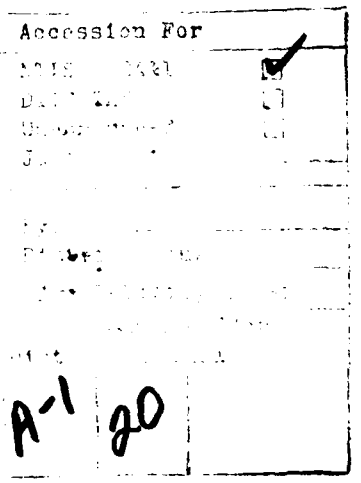

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